



Full Length Article

Influence of Bacterial Inoculation on Photosynthetic Capacity, Mineral Uptake, Total Protein and Amino Acid Content of Maize (*Zea mays*) under Diesel Stress

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Abstract

This study was undertaken to explore the influence of diesel contaminated soil and bacterial consortium on photosynthetic efficiency, ion uptake, chlorophyll fluorescence, chlorophyll pigments, amino acids and total protein content of maize crop. The experiment was consisted of (a) two maize varieties (MMR1 yellow and Pearl white), (b) diesel levels (0, 1.5, 2.5, 3.5 g kg⁻¹ soil) and (c) two bacterial strains inoculation levels, (non-inoculated) and inoculated with *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. ACRH80 and *Acinetobacter* sp. BRSI56 with completely randomized design having four replication. The results exhibited that diesel contaminated soil decreased photosynthetic efficiency, photosynthetic pigments i.e., 'a', 'b', carotenoids, chlorophyll fluorescence and k⁺ content, however, there is increase in amino acid accumulation, total protein and Na⁺ uptake. But inoculation of different bacterial strains enhanced the photosynthetic capacity of PSII, quantum yield (Fv/Fm), K⁺ uptake as compared to non-inoculated group along with hydrocarbon stress. The marginal lands contaminated with diesel oil could be ameliorated with inoculation of bacterial strains, which are highly adapted to various farming systems. The maize crop could be successively grown on polluted soil which are contaminated with poly aromatic hydrocarbons. The small holding farming families will be greatly benefited to improve their crop yields. © 2021 Friends Science Publishers

Keywords: *Pseudomonas aeruginosa* BRR154; *Acinetobacter* sp. ACRH80; *Acinetobacter* sp. BRSI56; *Zea mays* L.; Diesel stress; Photosynthetic capacity; Ion uptake

Introduction

The soil pollution has been considered as the “hidden danger” and a major threat to agricultural land and ecosystem across the continents (Rodríguez-Eugenio *et al.* 2018). The soil pollution is caused by petroleum derived products, industrial byproducts, livestock agrochemicals and municipal waste (Cachada *et al.* 2018). It does not degrade soil but also reduces crop yields as well as the production of products which is unsafe for consumption by humans and livestock. These pollutants cause significant harm to soil microflora, soil biodiversity and destroying the beneficial microbial population (Hentati *et al.* 2013; Mair *et al.* 2013; Baldan *et al.* 2015; Ramadass *et al.* 2015). The various plant species have differential tolerance levels to these organic pollutants (Aisien *et al.* 2009), because physiological processes of plants including photosynthetic efficiency, nutrients uptake and water relations (Iwegbue *et al.* 2007; Ibemesim 2010; Ambreen *et al.* 2016), photosystem II (PSII) (Li *et al.* 2010; Wang *et al.* 2012; Agnello *et al.*

2016) and chlorophyll contents (Li *et al.* 2008; Njoku *et al.* 2009) are the ones which are the worst affected in response to hydrocarbon contaminated soil.

The anthropogenic and mechanical error is responsible for the pollution of terrestrial land with petroleum hydrocarbons. The petroleum products spreading into different parts of the environment including soil and water due to accidental spillage worldwide, however, the exact data of petroleum hydrocarbons pollution is a hard task to present due to unintentional contamination. In Pakistan, in July 2003 a Greek ship (Tasman Spirit) cracked and resulting in 28,000 tons of crude oil accidentally polluted Karachi coastal line area. In September 2009 one more incident of an accidental spill of 18 crude oil tankers occurred in Sindh, Pakistan as a result of crashing two freight trains (Khan 2009). Another incident in Korangi Town Karachi, Pakistan was that the residential of the area found oil in their houses, streets, and shops because of destruction in the pipeline of Pak Arab Refinery Corporation (PARCO) while working of underground

construction by Sui Southern Gas Company (Alam 2008). The big oil spillage incident of two NATO oil tankers having 60,000 liters of gasoline collapsed on Khojak Pass near Chaman, Pakistan. Moreover, petroleum hydrocarbon contamination happened during the extraction of crude oil at well places, and petroleum refinery industries. The industries of petroleum refining in Pakistan are situated in Multan, Kot Adu, Qasaba Gujrat, Rawalpindi, and Karachi are the origin of contamination of oily residues sulphides, phenols, and hydrocarbons (NEPP 1999).

The extremely intricate and lethal petroleum hydrocarbons contaminate the surroundings though, complicates elimination. The conventional method (excavation, incineration, supercritical fluid oxidation and encapsulation, thermal desorption) are used for the cleanup of polluted sites (Peng *et al.* 2009; Khan *et al.* 2014) whereas these pollutants are necessary for immediate elimination and to constrain oil dispersal (Dixit *et al.* 2016). However, these methods exhibited demerits including high cost because of fuel consumption and less ecofriendly to environment and people (Kaimi *et al.* 2006). The eradication of petroleum hydrocarbons from the environs is done by an effective approach of phytoremediation (Pilon-Smits 2005; Afzal *et al.* 2012) Bioaugmentation is a promising strategy that remediates or detoxifies petroleum hydrocarbons with particular enzymatic characteristics by stimulating hydrocarbons microbial decontamination (Yousaf *et al.* 2010; Afzal *et al.* 2013; Sessitsch *et al.* 2013; Afzal *et al.* 2014; Souza *et al.* 2014; Hou *et al.* 2015; Xun *et al.* 2015; Fatima *et al.* 2016; Shahzad *et al.* 2016; Zhang *et al.* 2016; Ummara *et al.* 2020). Consequently, Plant and microbe are used together for detoxification of recalcitrant hydrocarbon pollutants (Jabeen *et al.* 2016; Guo *et al.* 2017).

Bacterial inoculation of *Streptomyces* sp. (Palaniyandi *et al.* 2014) in tomato and *Burkholderia phytofirmans* (Naveed *et al.* 2014) in maize alleviated the stress condition and subsequently enhanced biomass production. Under stress condition, in *Brassica juncea* inoculation (*Trichoderma harzianum*) mitigated Na⁺ uptake whereas progressed nutrient up take, acquisition of antioxidant and osmolytes (Ahmad *et al.* 2015). The ACC deaminase activity and production of indole acetic acid (IAA) were ameliorated by inoculation of *Pseudomonas* sp. and *Acinetobacter* sp. in barley and oats under stress condition (Chang *et al.* 2014). Plant growth regulator directly enhanced by microbes including nitrogen fixation, Fe and Zn sequestration, K and P solubilization, phytohormone and siderophore production (Almaghrabi *et al.* 2013; Meena *et al.* 2017).

There are evidences that various bacterial strains could be utilized to degrade PAHs. Therefore, the research studies were undertaken to quantify the efficiency of the isolated bacterial strains to degrade the hydrocarbons and to determine their effects on maize crop. A vast polluted waste land is lying around high ways

which could be utilized to grow cereal crops after degrading with bacterial strains. This research provides valuable understanding concerning physiological and biochemical effects of diesel contaminated soil and bacterial consortium on maize crop which could be used to subsistence the bioremediation strategy.

Materials and Methods

Experimental design

The pot-culture experimental studies were undertaken at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan-Pakistan. For two maize crop seasons (October–November 2017 and March–April 2018). The treatments were consisted of two maize varieties (MMRI - Yellow and Pearl- white) (b), four regimes of diesel (0.0,1.5,2.5,3.5 g /kg soil) and (c) two bacterial strains inoculation levels, (non-inoculated) and inoculated with *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. ACRH80 and *Acinetobacter* sp. BRSI56. The treatments were arranged in a three factor factorial with completely randomized design and each treatment was repeated four times. The strains *P. aeruginosa* BRR154 and *Acinetobacter* BRSI56 were isolated from the root and shoot of *Brachiaria mutica*, respectively, whereas *Acinetobacter* sp. strain ACRH80 was isolated from the rhizosphere of *Acacia ampliceps* (Fatima *et al.* 2015). These bacteria were capable to degrade a range of hydrocarbons having alkane hydroxylase gene (such as *alkB*) and exhibiting plant growth-promoting characteristics (phosphate solubilization, siderophore production, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity) were previously characterized and reported by Fatima *et al.* (2015).

Seed inoculation

Bacterial strains were grown in LB broth at 30°C on a shaker (100 rpm). The LB broth medium used for the culture of bacterial strains was harvest by centrifugation at 10,000 rpm for 10 min. Further washed and resuspended in sterilized normal saline solution (0.9% NaCl, w/v). Then seed and soil inoculation were done by using bacterial suspension (Afzal *et al.* 2012; Ummara *et al.* 2020). Before sowing, the seeds were surface-sterilized in a 5% (v/v) NaOCl for 10 min, washed three times with sterilized distilled water. After sterilizing, the seeds were dipped in the prepared bacterial inoculum (10⁸ cfu mL⁻¹) for 2 h and numeration of bacterial inoculants in the rhizosphere soil, shoot and root samples collected at the time of harvest was checked and reported in latest study (Ummara *et al.* 2020). The pots were arranged in a green house in a completely randomized block designed. The plastic pots were filled with 6 kg of soil and contaminated with various levels of commercial disease. Five plants were maintained in each pot by thinning at 7 days after germination.

Estimation of chlorophyll (Chl) content and chlorophyll (Chl) fluorescence

The estimation of Chl a, b, total Chl, carotenoids were carried out as previously described with certain modifications (Aronoff 1946). Briefly, 0.2 g of the fresh leaf was grinded in 80% acetone and absorbance was read at 663 nm, 645 nm, and 470 nm. Photosynthetic efficiency parameters (Y(II), Fv/Fm, NPQ) were determined using a Dual-PAM 100 (Heinz Walz, Effeltrich, Germany) subsequently maize was adapted to the dark for 30 min, where YII (PSII quantum yield effectiveness), Fv/Fm (maximum quantum yield of PSII after dark adaptation), NPQ (PSII photo-inactivation and constitutive heat dissipation).

Estimation of total soluble proteins and total free amino acid (TPS and TAA)

TPS was quantified by using bovine serum albumin (BSA) as a standard procedure with some modification (Bradford 1976). 0.2 g of fresh leaf tissue was homogenized in phosphate buffer (pH 7.8, 4 mL) and centrifuged at 10,000×g. TAA content was quantified by using the following protocol (Hamilton *et al.* 1943). The quantification was done by treating the sample with 2% ninhydrin (1 mL) and 10% pyridine (1 mL). The absorbance was taken at 570 nm and leucine as used as a standard.

Estimation of Na⁺ and K⁺

For the estimation of Na⁺ and K⁺ ions, dry sample (leaf and root, 0.1 g) was digested in 2 mL of sulfuric- peroxide digestion mixture (Wang and Zhao 1995). Then deionized water was used to maintain the volume (50 mL) of each sample. The estimation of Na⁺ and K⁺ ion analysis was done by using a flame photometer.

Gas exchange attributes

For the measurement of leaf gas exchange attributes comprising, *A*, *E*, *A/E*, *gs*, and *Ci* using an open system Ci-340 portable photosynthesis system (CID, USA). The measurements were made from 10:00 am to 14:00 pm with the subsequent amendments: leaf surface area 6.3 cm², ambient CO₂ concentration 385 μmol mol⁻¹, temperature of leaf chamber fluctuating from 40 to 43°C, leaf chamber volume gas flow rate (*v*) 1068 mL min⁻¹, leaf chamber molar gas flow rate (*U*) 1052 μmols⁻¹, ambient pressure (*P*) 100.09 kPa and PAR (*Q* leaf) at leaf surface maximum up to 874 μmol m⁻² s⁻¹.

Statistical analysis

SPSS software package (SPSS In., USA.) and Excel (Microsoft, U.S.A.) was used to perform statistical analyses.

Duncan's multiple range tests were applied for the analysis of variance and LSD between means of data.

Results

Chlorophyll constituents

The quantum of chlorophyll constituents i-e., 'a', 'b', total chlorophyll and carotenoids contents were degraded significantly ($p < 0.001$) with each increment of diesel oil stress in both varieties viz., "MMRI Yellow" and "Pearl White" of maize crop (Table 1). However, the values of chlorophyll pigments were significantly increased in maize plants grown when the soil was inoculated with bacterial strains as compared to the non-inoculated growth medium. The contents of chl. 'a', 'b', total chlorophyll and carotenoids were increased by quantum of 32.3%, 59.8%, 40.9%, 31.9%, respectively in var. MMRI Yellow grown on inoculated growth medium over the non-inoculated soil condition. On the other hand, var. Pearl White grown on inoculated growth medium contained lower values of chl, a, b, total chlorophyll, and carotenoids by an amount of 27.6%; 56.5%; 36.8%; 9.8%, respectively compared to non-inoculated soil environment.

Chlorophyll fluorescence attributes (Y (II), Fv/Fm, NPQ)

The values of attribute Y(II) were reduced significantly ($P < 0.001$) in crop plants on growth medium contaminated with 3.5 g kg⁻¹ diesel oil (Fig. 1A). However, the content of Fv/Fm was substantially increased in maize crop growing on inoculated soil medium (Fig. 1B). The var. MMRI Yellow maintained higher content of Fv/Fm by 6.67% compared to Var. Pearl White, having an amount of 5.77% under inoculated growth medium as compared to non-inoculated soil environment. Furthermore, the values of NPQ were enhanced significantly ($P < 0.001$) by growing crop plants on growth medium inoculated with bacterial consortium. Under the inoculated growth medium, var. MMRI-Yellow contained lower value of NPQ by 2.92%, contrarily to var. Pearl White having higher value of 4.2% as compared to non-inoculated growth condition (Fig 1C).

Gas exchange attributes

The data of the net CO₂ assimilation rate (*A*) was differed significantly ($P < 0.001$) in response to various diesel regimes, inoculating growth medium with bacterial strains and crop varieties. The values of *A* decreased substantially in crop plants with increasing levels of hydrocarbons. The crop treated with 3.5 g kg⁻¹ diesel oil maintained minimal amount of net Photosynthetic rate (Fig. 2A). In concurrence with increasing levels of hydrocarbons, to the tune of 3.5 g kg⁻¹ diesel oil, the transpiration rate (*E*) was reduced to the where minimums levels (Fig. 2B). However, its values were appreciably enhanced in crop grown on bacterial consortium

Table 1: Influence of varying diesel regimes on chlorophyll contents of *Zea mays* L. cultivated for 4 weeks with and without bioaugmentation of *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. BRS156, *Acinetobacter* sp. ACRH80

Varieties	Treatment	Hydrocarbon Concentration (g kg ⁻¹ soil)	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
MMR1 yellow	Bacterial Consortium	0	0.51 ±0.00a	0.28 ±0.00a	0.80 ±0.01a	0.37 ±0.00a
		1.5	0.33±0.01b	0.17 ±0.02b	0.51±0.02b	0.25 ±0.01b
		2.5	0.31±0.00c	0.15 ±0.01bc	0.46 ±0.01b	0.20 ±0.01c
		3.5	0.28±0.00c	0.11 ±0.01c	0.40 ±0.01c	0.18 ±0.01c
	Without Bacterial consortium	0	0.38 ±0.00a	0.17 ±0.01a	0.57 ±0.01a	0.28 ±0.01a
		1.5	0.31 ±0.00b	0.13 ±0.02b	0.43 ±0.00b	0.19±0.01b
		2.5	0.25 ±0.01c	0.12 ±0.00b	0.38 ±0.03b	0.16 ±0.01c
		3.5	0.17 ±0.00d	0.10 ±0.00b	0.28 ±0.00c	0.14 ±0.01c
Pearl White	Bacterial Consortium	0	0.45 ±0.01a	0.25 ±0.00a	0.71 ±0.01a	0.30 ±0.00a
		1.5	0.32 ±0.00b	0.14 ±0.00b	0.46 ±0.01b	0.21 ±0.01b
		2.5	0.29 ±0.00c	0.13 ±0.01b	0.43 ±0.01c	0.19 ±0.01b
		3.5	0.27±0.00d	0.11 ±0.01c	0.38±0.01d	0.16 ±0.01c
	Without Bacterial consortium	0	0.35 ±0.01a	0.16 ±0.00a	0.52 ±0.01a	0.27±0.01a
		1.5	0.28 ±0.00b	0.12 ±0.01b	0.40 ±0.01b	0.18 ±0.01b
		2.5	0.22 ±0.00c	0.10 ±0.01bc	0.32 ±0.01c	0.15 ±0.01c
		3.5	0.15 ±0.00d	0.09±0.01c	0.25 ±0.01d	0.14 ±0.01c

Values express in terms of means of four replicates ± SE. According to Duncan's multiple range test, dissimilar letter representing means are significant statistically (P < 0.05)

inoculated soil. The growth medium contaminated with diesel oil caused significant ($p < 0.001$) reduction in water-use-efficiency (A/E). The greatest reduction of A/E was recorded in crop grown on soil containing 1.5 g kg⁻¹ diesel oil, while, its values were found to be improved by growing both crop varieties on soil contaminated with 3.5 g kg⁻¹ diesel stress (Fig. 2C). The occurrence of have relative rate of Transpiration due to inoculation of growth medium with bacterial consortium resulted in changing stomatal conductance under diesel polluted stress environment. The values of stomatal conductance (g_s) and sub-stomatal CO₂ (C_i) concentration were increased significantly in maize plants grown on growth medium inoculated with bacterial consortium as compared to non-inoculated growing conditions. However, values of these attributes were found in decreasing order with increasing concentration of diesel oil in growth medium (Fig 2D and E).

Assimilation of Ionic Constituents by Leaves and Roots Tissue

The application of diesel oil in root growing medium caused increase in assimilation of Na⁺ ion by shoot and root organs of both maize varieties compared to non-hydrocarbon stress conditions. However, inoculation of growth medium with bacterial consortium cause substantial reduction in the uptake of Na⁺ ion compared to non-inoculated growing condition. The maize varieties responded differently in the absorption of Na⁺ growing under inoculated growing medium. In proportion to total amount of Na⁺, its absorption was decreased by var. MMRI Yellow and Pearl White to the tune of 13.08% and 11.31% respectively, in inoculated crop over the non-inoculated growth condition (Fig. 3C and D).

Successive increase in the levels of diesel stress caused sequential reduction in uptake of K⁺ ion by shoot and root organs in both maize varieties. The uptake of K⁺ ion was substantially improved by growing crop on growth medium inoculated with bacterial consortium. Var. "MMRI Yellow"

assimilated higher amount of K⁺ ion by 30.46% as compared to 29.73% by var. "Pearl White" once (Fig. 3A and B).

Total soluble protein and total free amino acid contents

Addition of various concentration of diesel oil resulted in enhancement of total soluble proteins in root and shoot tissues of both varieties. The greater quantity of total soluble protein content were recorded in crop stressed at 3.5 g kg⁻¹ as compared to non-polluted soil. Moreover, crop grown on soil enriched with bacterial consortium maintain higher amount total soluble protein in comparison with non-inoculated growing environment (Fig. 4A, B). The values of total free amino acids were increased substantially in crop grown on diesel contaminated growth medium. Moreover, its value were also enhanced in root and shoot tissue of crop planted on soil inoculated with bacterial consortium. The maximum amount of total free amino acids were recorded in crop grown on soil contaminated with 3.5 g kg⁻¹ as compared to other growth medium added with 1.5 and 2.5 g kg⁻¹ diesel stress (Fig. 4C and D).

Discussion

The sustainability of chlorophyll constituents, gas exchange and pattern of uptake of ions were studied under hydrocarbon stress environment in maize crop. The findings of regarding physiological processes revealed that both maize varieties exhibited significantly greater production of quantum yield (Fv/Fm, photosystem II efficiency [Y(II)], high uptake of K⁺ ions and CO₂ assimilation rate, total free amino acid, and total soluble proteins; while lessening non-photochemical quenching (NPQ), and transpiration rate, in response to inoculation of growth medium with bacterial strains. The plants growing on inoculated medium showed greater tolerance to hydrocarbon treated soil compared to un-inoculated environments.

The present investigation illustrated that inoculation of growth medium with bacterial strains produce beneficial

effects in the environment of photosynthetic pigments, Fv/Fm and photosystem II efficiency in plants under hydrocarbon stress. Ambreen *et al.* (2016) also reported same result in corn plant under crude oil stress. Another investigate supported our result by Agnello *et al.* (2016) in which *Medicago sativa* grown in diesel and heavy metal (Cu, Pb and Zn) contaminated soil and bioaugmentation with *Pseudomonas aeruginosa* showed better result of quantum yield of photosystem II (Fv/Fm) in inoculated group as compare to non-inoculated group.

The chlorophyll synthesis was greater affected at a high concentration of hydrocarbon, while it was a little at the lower concentrations. The quantum of chl 'a' and chl 'b' content were not damaged to a greater proportion in maize plants in bacterial inoculated group. The degeneration of photosynthetic pigments might be affected due to membrane permeability and disturbance in the chloroplast. The results of our study showed that toxicity caused by hydrocarbon resulted in the diminishing of chloroplast function, which led to damage to the chlorophyll contents. However, these damaging effects were mitigated by inoculation with bacterial strains of hydrocarbon contaminated soil. The hydrocarbon stress caused significant damaging effect to the chlorophyll contents evidenced by Agnello *et al.* 2016 and Ambreen *et al.* 2016. However, *Oryza sativa* grown in phenanthrene and pyrene contaminated soil badly affects photosynthetic efficiency by causing disintegration of chlorophyll contents but bacterial inoculation (*Acinetobacteria* sp.) mitigated effects of phenanthrene and pyrene in rice plant also reported by Li *et al.* 2008.

Results of our investigation showed that stomatal conductance and sub-stomatal CO₂ concentration of both maize varieties were improved due to bacterial inoculation. Moreover, transpiration rate (*E*) of both varieties were enhanced in inoculated group as compare to non-inoculated group. The leaves of hydrocarbon stressed maize varieties exhibited higher water use efficiency (WUE) as compare to control. Iwegbue *et al.* (2007) reported that the photosynthetic processes were inhibited, because of a reduction in net CO₂ assimilation rate, transpiration rate, sub stomatal CO₂ concentration and coupled with high ambient CO₂ concentration. However, stomatal closure produces little effect on the process of photosynthesis. During the process, the light energy is captured by photosynthetic pigment to produce an excited state and contaminated stress was a marker to demonstrate a sign of plant photosynthetic efficiency (Qian *et al.* 2014). Whereas, other abiotic and biotic stresses also cause to reduce photosynthetic pigments and photosynthetic efficiency in plants (Wen *et al.* 2016; Li *et al.* 2018).

The results of our study documented carotenoids severely damaged by hydrocarbon stress in maize varieties grow in diesel contaminated soil. Ibemesim 2010 reported that sour grass grown in crude oil contaminated soil badly affects photosynthetic efficiency by causing disintegration of chlorophyll contents. Carotenoids act as antioxidants as

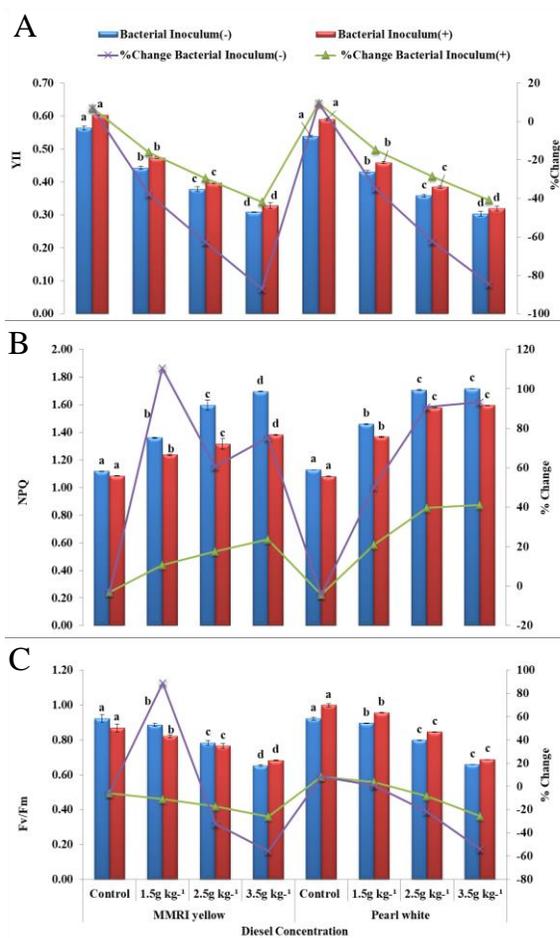


Fig. 1: Influence of varying diesel regimes on chlorophyll fluorescence attributes (Y (II), Fv/Fm, NPQ) of *Zea mays* L. Cultivated for 3 weeks with and without bioaugmentation of *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. BRS156, *Acinetobacter* sp. ACRH80. (-) and (+) signs indicate that without inoculum and with inoculum

scavenge to free radicals, mitigating destruction of cells and avoiding damage to chlorophyll membrane when stressful conditions (Czerpak *et al.* 2006). Thereby, the quantum of chlorophyll content, biosynthesis of photosynthetic pigments, mineralization of mineral nutrients and essential metabolites were substantially affected by toxicity caused by hydrocarbons (Ibemesim 2010).

Our study showed that the inoculation of growth medium with bacterial strains resulted in reduced uptake of Na⁺ ions by leaves and roots of maize plants. The reduction in absorption of Na⁺ led to the improvement in plant growth and development by enhancing tolerance in the plant system. However, our investigation revealed that the uptake of K⁺ ion was also accelerated with simultaneously suppressing the uptake of Na⁺ ion under inoculated conditions. The previous investigation by Ahmad *et al.* 2015 reported the similar results that bacterial inoculation (*Trichoderma harzianum*) alleviated the abiotic stress in *Brassica juncea*.

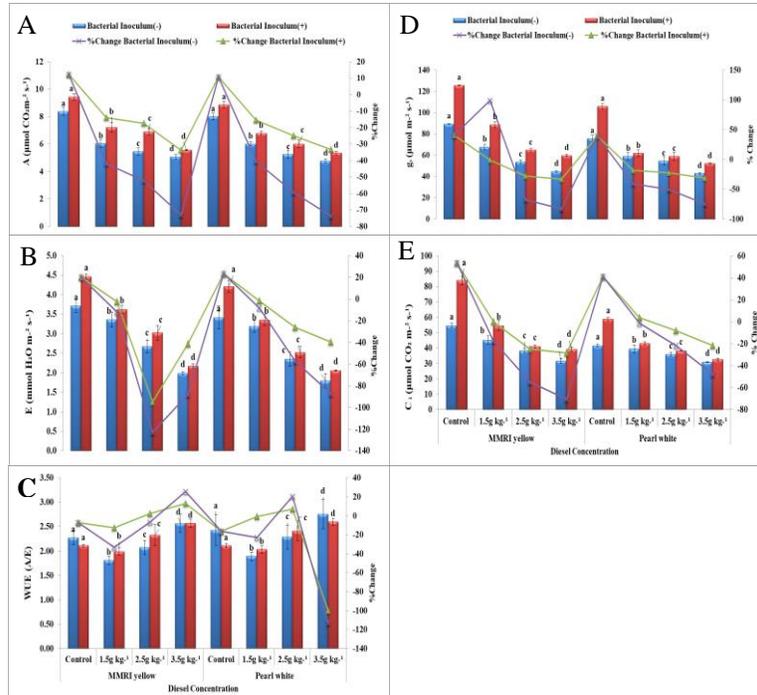


Fig. 2: Influence of varying diesel regimes on gas exchange attributes (A, B, C, D, E) of *Zea mays* L. cultivated for 6 weeks with and without bioaugmentation of *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. BRS156, *Acinetobacter* sp. ACRH80. (-) and (+) signs indicate that without inoculum and with inoculum

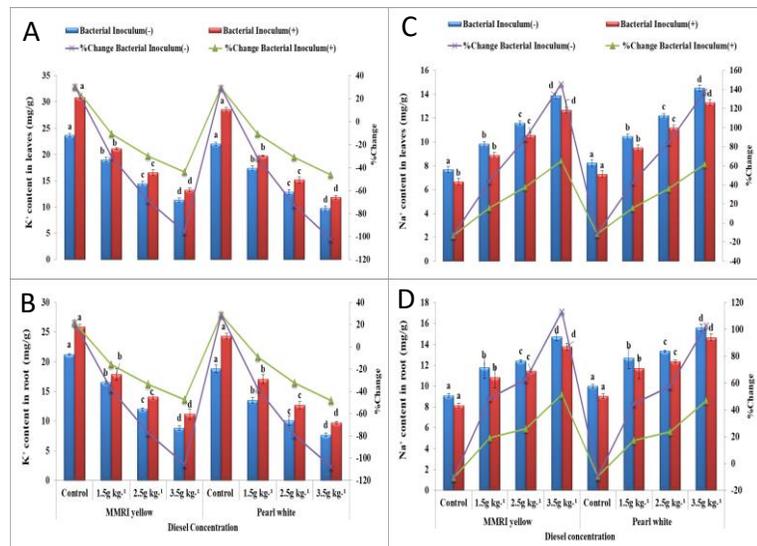


Fig. 3: Influence of varying diesel regimes on K⁺ (A and B) and Na⁺ (C and D) uptake in the leaf and root tissues of *Zea mays* L. Cultivated for 6 weeks with and without bioaugmentation of *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. BRS156, *Acinetobacter* sp. ACRH80. (-) and (+) signs indicate that without inoculum and with inoculum

Zhuang *et al.* (2007) revealed that inoculation of plant growth promoting bacteria enhanced the process mineralization and providing better condition for growth and development of the plant by reducing the effects of stressful environment. The inoculation of growth medium induces mineralization of minerals nutrients and

creating a favourable environment for greater absorption of nutrients by plant roots (Meena *et al.* 2017).

The increased accumulation of total free soluble proteins and total free amino acids play a significant role in the maintenance of physiological functions in response to stressful conditions during the growth period

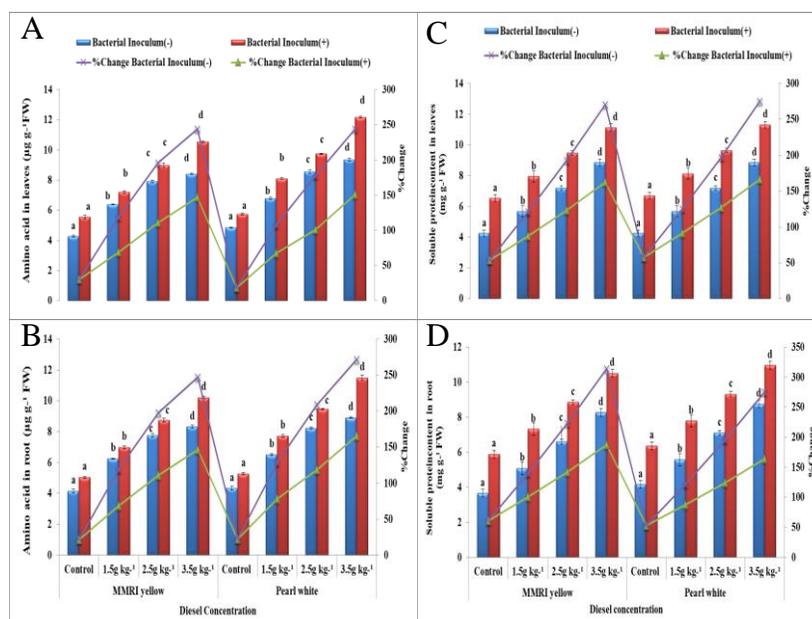


Fig. 4: Influence of varying diesel regimes on total free amino acids (A and B) and total soluble protein (C and D) of *Zea mays* L. cultivated for 4 weeks with and without bioaugmentation of *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. BRS156, *Acinetobacter* sp. ACRH80. (-) and (+) signs indicate that without inoculum and with inoculum

(Ashraf and Harris 2004). The results of our study also revealed that quantum of total free amino acids and total soluble proteins also increased under stressful conditions of hydrocarbon present in the rhizosphere in maize crop. Ambreen *et al.* 2016 also reported that maize plant grown in crude oil contaminated soil showed increased amount of total soluble proteins and total free amino acid. Li *et al.* 2008 also reported that rice plant grown in polycyclic aromatic hydrocarbon contaminated soil showed enhanced level of total soluble proteins. The existence of a potential association between plants and microbes is important in degrading hydrocarbon substances from the soil (Afzal *et al.* 2012).

The bacterial degrade the complex structures of hydrocarbons and convert into soluble forms. Thereby, the nutrients are made available and easily absorbed by plant roots, restoring to improving tolerance to stress conditions (Muratova *et al.* 2012; Hou *et al.* 2015; Gerhardt *et al.* 2017a; Gerhardt *et al.* 2017b).

Moreover, vegetation covers also provide the habit for microbes to burgeon their population in the vicinity of roots in the rhizosphere (Susarla *et al.* 2002). The microbial populations in the rhizosphere do not only degrade substances of hydrocarbons but also convert other crop residues and farmyard manure into soluble substance. The products work as biofertilizers, psychostimulants and creating a favorable environment for better growth and development. The prevalence of a better rhizosphere microenvironment for plant roots resort to a greater proliferation of microbial activities (Braud *et al.* 2009; Hayat *et al.* 2010).

Conclusion

A pot-culture study was undertaken to quantify the association of bacterial consortium to degrade the diesel polluted soil. The cereal maize (*Zea mays* L.) crop was used as test plant. The findings of this research indicated that contamination of soil with PAHs impacted negatively on the growth and development of maize crop. The maximum concentration of 3.5 g kg⁻¹ diesel oil proved to be more injurious on various physiological and biochemical attributes. The negative effects of polluted soil could be ameliorated by inoculation of growth medium with bacterial consortium, viz. *Pseudomonas aeruginosa* BRR154, *Acinetobacter* sp. ACRH80 and *Acinetobacter* sp. BRS156. The soil inoculated with bacterial strains caused improvement in photosynthetic machinery, assimilation of K⁺ at the cost of Na⁺ ion by plant organs and osmotic adjustment via accumulation of total proteins and amino acids.

The preliminary experimental evidence has shown that inoculation of soil with various bacterial strains that hold good in the different eco-edaphic environment could be best utilized to bring back hydrocarbon polluted soil under plough for development agro-forestry sector. The small farming households would be greatly benefited to bring their marginal lands for cultivation of maize crop. The bacterial strains employed in this study were isolated from the natural habitat. Therefore, these strains would have an equal opportunity to survive in different farming system. The finding of this study could be extrapolated under field condition. Moreover, the research and endeavors could be

strengthen to isolate more environment friendly bacterial strains, that could be more efficient and greater capability to survive for degradation of diesel polluted soil.

Authors Contributions

UU, SN, ZUZ and MA designed the experimental setup and UU conducted the experiments. UU and SN wrote the manuscript. UU analyzed the data. SN critically revised the manuscript to the current form.

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